Coupled Biogeochemical Process Evaluation for Conceptualizing Trichloroethylene Co-Metabolism

Frederick Colwell, Corey Radtke, Deborah Newby, and Mark Delwiche (Idaho National Laboratory)
Ronald L. Crawford, Andrzej Paszczynski, and Janice Strap (University of Idaho)
Mark Conrad and Eoin Brodie (Lawrence Berkeley National Laboratory)
Robert Starr and Hope Lee (North Wind, Inc.)

Abstract

Chlorinated solvent wastes (e.g., trichloroethene or TCE) often occur as diffuse subsurface plumes in complex geological environments where coupled processes must be understood in order to implement remediation strategies. Monitored natural attenuation (MNA) warrants study as a remediation technology because it minimizes worker and environment exposure to the wastes and because it costs less than other technologies. However, to be accepted MNA requires "lines of evidence" indicating that the wastes are effectively destroyed. Our research will study the coupled biogeochemical processes that dictate the rate of TCE co-metabolism in contaminated aquifers first at the Idaho National Laboratory and then at Paducah or the Savannah River Site, where natural attenuation of TCE is occurring. We will use flow-through in situ reactors to investigate the rate of methanotrophic co-metabolism of TCE and the coupling of the responsible biological processes with the dissolved methane flux and groundwater flow velocity. We will use new approaches (e.g., stable isotope probing, enzyme activity probes, real-time reverse transcriptase polymerase chain reaction, proteomics) to assay the TCE co-metabolic rates, and interpret these rates in the context of enzyme activity, gene expression, and cellular inactivation related to intermediates of TCE co-metabolism. By determining the rate of TCE co-metabolism at different methane concentrations and groundwater flow velocities, we will derive key modeling parameters for the computational simulations that describe the attenuation, and thereby refine such models while assessing the contribution of microbial relative to other natural attenuation processes. This research will strengthen our ability to forecast the viability of MNA at DOE and other sites that are contaminated with chlorinated hydrocarbons.

Introduction

Our objectives are to: 1) determine the controls on TCE co-metabolism rates by quantifying the coupled biogeochemistry (e.g., methane production and consumption) and hydrology (e.g., rate of fluid movement in primary flow paths) using in situ mesoscale reactors and 2) derive the enzyme activities of cells that are performing TCE co-metabolism in order to determine the relationship between the expression levels of key genes related to TCE co-metabolism, the presence of a broader array of proteins, and to the actual TCE transformation rates.

Using our approach we expect to obtain an improved conceptual model of natural attenuation at DOE sites and refine site-specific computational models of natural attenuation. We also expect to determine how the molecular characteristics of cells performing the TCE co-metabolism are coupled to the hydrological conditions and the methane flux in which the cells exist.

Methods

The field site for our research is the TCE-contaminated aquifer at Test Area North (Fig. 1) at the Idaho National Laboratory where monitored natural attenuation of TCE has been accepted as a remediation strategy. Methanotrophs and methanogens exist in the aquifer (Fig. 2) (Erwin et al. 2005; Newby et al. 2004) and the methanotrophs apparently contribute to the co-metabolism of TCE (Lee et al. in revision).

To determine how key characteristics of the methanotrophs respond to critical abiotic features in the aquifer (e.g., aquifer flow rates, methane concentrations), we will use a flow-through in situ reactor (FTISR) (Fig. 3) in well TAN-35 (Fig. 1) to control these abiotic factors. Basalt from the aquifer will be colonized in the FTISR over an extended period with flow rates of 1 m/s and 0.1 m/s. Subsequently, the FTISR will be recovered and the methanotroph and general microbial community characteristics in the water and basalt will be evaluated (Fig. 3). Follow-on lab experiments with the colonized basalt will allow us to determine the relationship between TCE co-metabolism rates and the community parameters under the different abiotic conditions. These data will also contribute to model development for monitored natural attenuation.

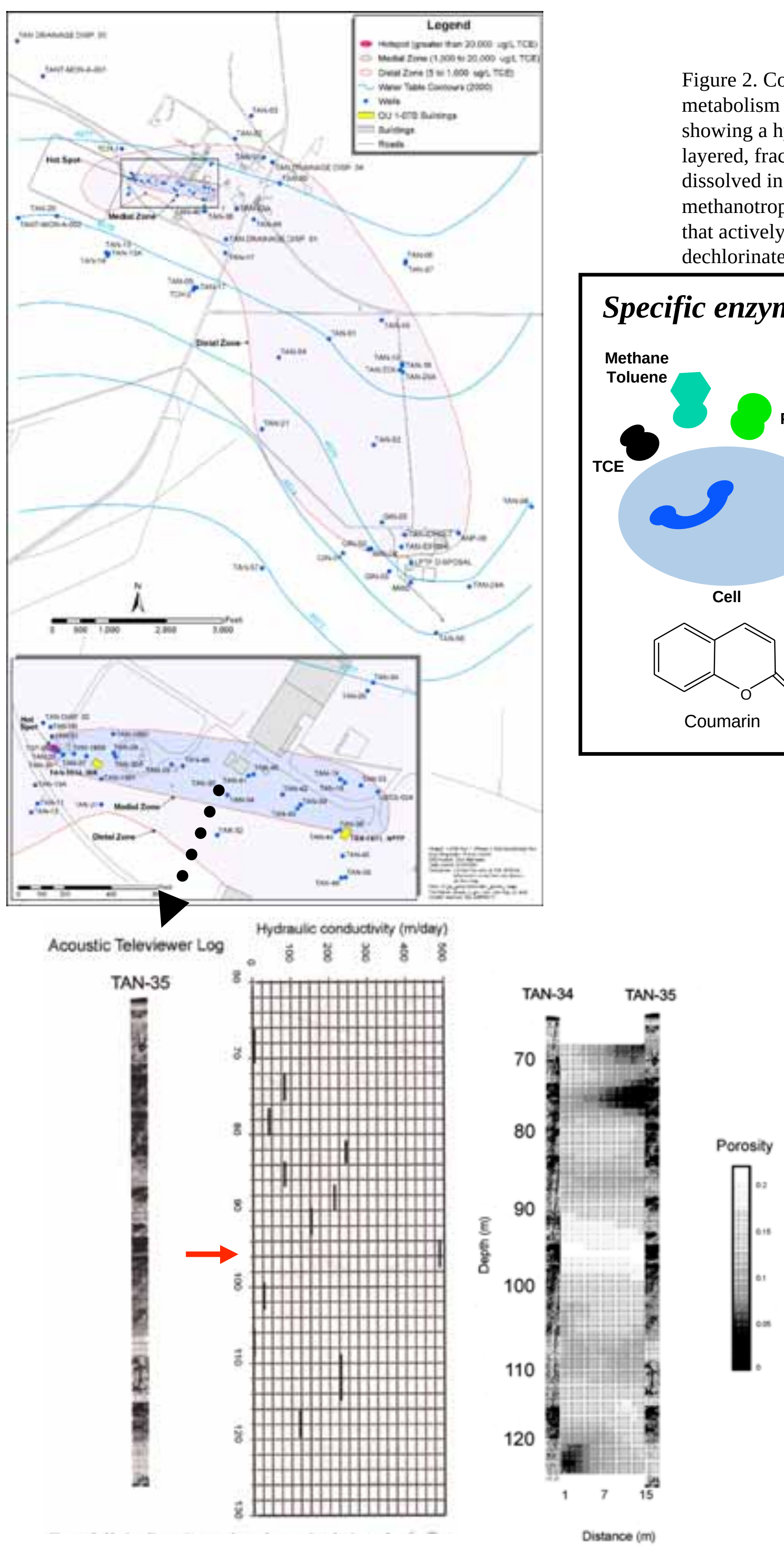


Figure 1. Location of TAN-35, the research well to be used in this investigation along with published data on the hydraulic conductivity and porosity determined by radar tomography (see Wylie et al. 2002 and Knoll and Lane, 1997). The high permeability zone is noted by red arrow.

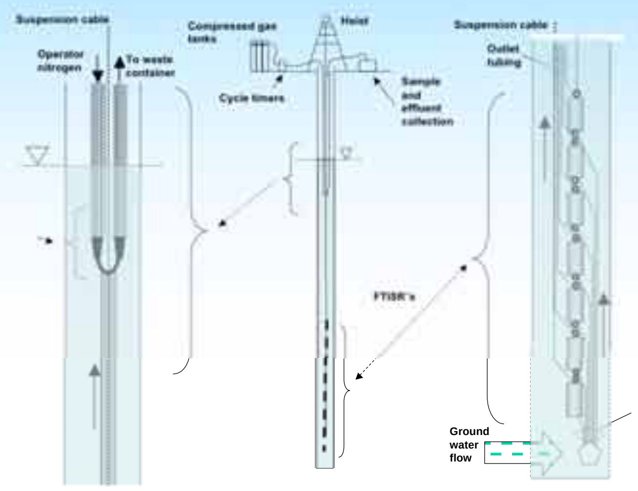
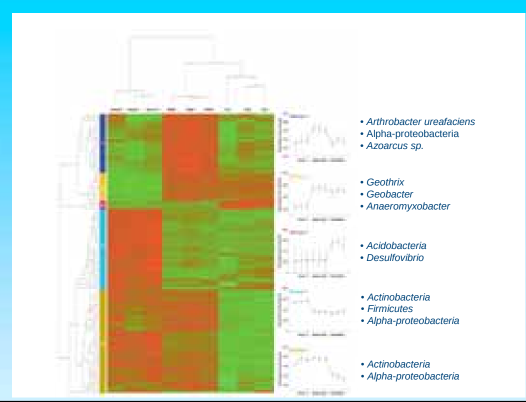


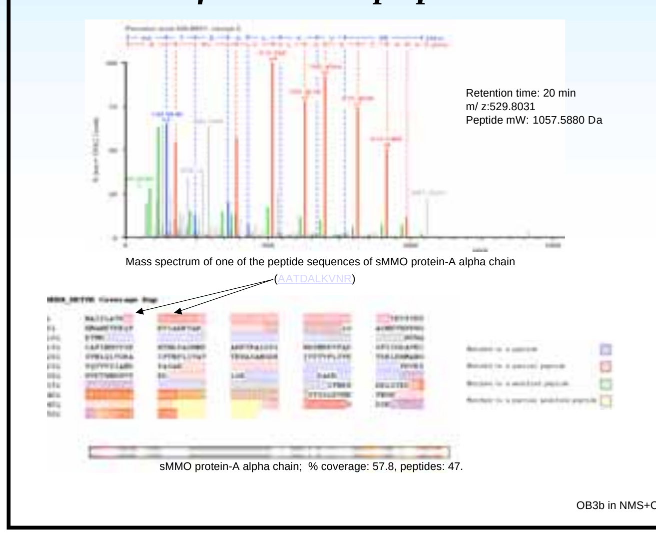
Figure 3. Diagram showing (six) FTISR reactors submerged in the groundwater (center; right). FTISR are connected in a string and suspended from land surface by a steel cable. Each reactor is 6.3 cm (internal diameter) x 120 cm long with 3700 cm^3 volume. Groundwater flow rate through each reactor is pneumatically controlled from the surface through "U" bends (left). Analytical methods and modeling approaches are shown around the FTISR diagram.

Figure 2. Conceptual model of TCE co-metabolism in the Snake River Plain aquifer showing a hypothetical cross-section of the layered, fractured basalts. Biogenic methane is dissolved in the groundwater and sustains methanotrophs, a key groundwater community that actively expresses the enzymes required to dechlorinate the TCE.

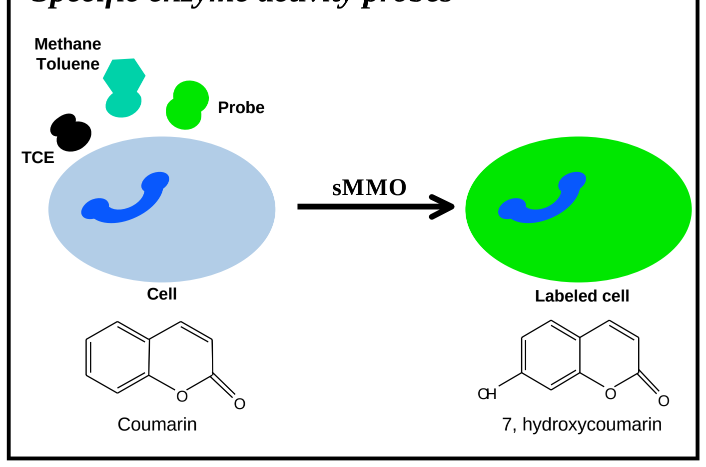
High density microarray for community profiling targeting 16S rDNA and rRNA



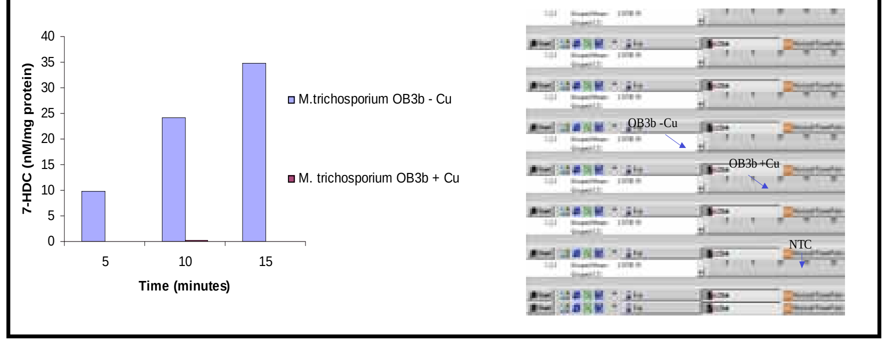
Detection of methanotroph proteins



Specific enzyme activity probes



sMMO activity (by coumarin oxidation activity) and expression of mmoX (by reverse transcriptase real-time PCR); methanotroph biomass by real time PCR



Stable isotopes ($^{13}CH_4$) to confirm biological methane oxidation, the oxidation rate, and to determine the fate of the carbon (bulk biomass, PLFA, nucleic acids), shifts of $\delta^{13}C$ of CH_4 and DIC

Data from these studies will be used to address the modeling objectives:

Determine which kinetic model(s) are appropriate for describing TCE cometabolism in a field-scale model.

Determine how to parameterize such a model using data that can be measured using field- or lab-based methods.

Determine the effect of various parameters on TCE transport.

Preliminary results and plans

The FTISR have been designed and a prototype is being constructed. Deployment of the completed FTISR assembly (Fig. 3) is scheduled for August 2006.

Microbes were filtered from water collected from TAN-35 and TAN-58 (a control well outside the TCE plume) in order to determine the presence of methanotrophs in these wells. Primers targeting the sMMO gene successfully amplified methanotroph DNA from filtered water samples collected TAN-33, -43, and -36. We hope to examine these samples with the phylochip technology to determine the presence of microbial community representatives other than methanotrophs.

DNA extraction procedures adapted for methanotrophs are being developed.

Pre-FTISR testing for development of analytical procedures:

1. Basalt substrates were lowered into TAN-35 and TAN-58 for extended incubation in order to obtain microbial-colonized basalt.
2. In early April, a cartridge containing basalt will begin to receive aquifer water augmented with methane in order to collect methane-enriched biomass.
3. Prototype FTISR will be tested in the aquifer in May-June timeframe.

Methylosinus trichosporium OB3b has been distributed to collaborators and is being used as a model methanotroph during studies to refine the methods which will interrogate the FTISR microbial communities. Preliminary results from the proteomics studies identified key methanotroph polypeptides in pure cultures of OB3b.

References

Erwin, D. P., I. K. Erickson, M. E. Delwiche, F. S. Colwell, J. L. Strap, and R. L. Crawford. 2005. Diversity of oxygenase genes from methane- and ammonia-oxidizing bacteria in the Eastern Snake River Plain aquifer. Appl. Environ. Microbiol. **71:**2016-2025.

Knoll, M. D., and J. W. Lane. 1997. Use of borehole-radar methods to characterize hydrostratigraphy in basalt. Presented at the Fall Meeting, American Geophysical Union, San Francisco.

Lee, M. H., W. K. Keener, R. Wymore, A. Miller, F. Colwell, K. S. Sorenson, and M. E. Watwood. Intrinsic aerobic TCE cometabolism by methanotrophs expressing sMMO: Detection of enzyme activity and tentative species identification. Environ. Sci. Technol. IN revision.

Newby, D. T., D. W. Reed, L. M. Petzke, A. L. Igoe, M. E. Delwiche, F. F. Roberto, J. P. McKinley, M. J. Whiticar, and F. S. Colwell. 2004. Diversity of methanotroph communities in a basalt aquifer. FEMS Microbiol. Ecol. **48:**333-344.

Wylie, A. H., D. R. Ralston, and G. S. Johnson. 2002. Effect of basalt heterogeneity on intrinsic bioremediation processes in groundwater, p. 287-296. *In* P. K. Link and L. L. Mink (ed.), Geology, hydrogeology, and environmental remediation: Idaho National Engineering and Environmental Laboratory, Eastern Snake River Plain, Idaho, vol. Special Paper 353. Geological Society of America, Boulder, CO.

Acknowledgements

Work was supported by funding from the U.S. Department of Energy, Office of Science, to the Idaho National Laboratory, operated by Battelle Energy Alliance, LLC, under contract DE-ACO7-05ID14517.

North Wind   Berkeley Lab   University of Idaho   Idaho National Laboratory